Hormonal contraceptives and venous thrombosis

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General introduction

Chapter 1

Introduction

Steroid hormone use and venous thrombosis

In 1960, shortly after the introduction of combined oral contraceptive Enovid ® (150 µg mestranol, an estrogen, and 985 mg norethynodrel, a progestagen), the first case of venous thrombosis associated with contraceptive use was reported[1]. Since then many observational studies have been conducted to assess the association between combined oral contraceptives and venous thrombosis. Overall, combined oral contraceptive use is associated with a two-fold to six-fold increased risk of venous thrombosis[2–5]. Nowadays many women worldwide use oral contraceptives making the impact of oral contraceptive use on venous thrombosis risk large, despite the low incidence of venous thrombosis of about 3 per 10,000 woman-years among women of reproductive age[6].

The causality between hormone use and venous thrombosis can be discussed using Hill's criteria of causality[7]. A review by Vandenbroucke et al[8] showed that the association between combined oral contraceptive use and venous thrombosis was consistent and of the same strength over several observational studies. The plausibility of the association is strengthened by the effect of oral contraceptives on the levels of several coagulation factors and the resulting shift in the balance of coagulation towards a prothrombotic state. However, the mechanism behind this effect remains unclear.

An outline of the current state of literature is given on the risk of venous thrombosis associated with hormone use and the biological mechanism that may explain the prothrombotic effect. First, the association between hormone use and the risk of venous thrombosis will be evaluated. The following applications of estrogens or progestagens will be addressed; contraception, relieve of menopausal symptoms, restriction of tall stature and sex change. Secondly, a hypothesis will be derived on whether estrogen, progestagen or combination of estrogen and progestagen may lead to venous thrombosis. Thirdly, the effect of hormone

use on coagulation factors, activated protein C resistance and sex hormone binding globulin levels will be evaluated. Lastly, the outline of this thesis with the research questions will be proposed.

Hormonal contraception

Hormonal contraception is a birth-control method to prevent ovulation and thus pregnancy. Hormonal contraception consists of steroid hormone use in two main types of formulations; combined formulations which contain both estrogen and progestagen and progestagen-only formulations. Progestagen suppresses the surge in luteinizing hormone (LH) and thereby prevents ovulation. Estrogen reduces the secretion of follicle-stimulating hormone (FSH) and thereby inhibits folliculogenesis. The estrogen compound has a major role in drug compliance; by increasing the stability of the endometrium, breakthrough bleeding and spotting are reduced. Hormonal contraception is prescribed to regulate the uterine, menstrual cycle or for other hormonally dependent disorders as acne[9] and hirsutism[10].

The most commonly used estrogen in combined hormonal contraceptives is ethinylestradiol, whereas different types of progestagens are used in combined or progestagen-only contraceptives. Contraceptive progestagens can be categorised according to the time of their introduction (first, second and third generation, respectively introduced in the sixties, seventies, and eighties of the last century) or according to their tetracyclic structure[11], i.e. estranes (derivatives of testosterone), pregnanes (derived from the progesterone molecule) and gonanes. The following progestagens correspond to first generation progestagens: norethisterone (NET), ethynodiol diacetate, lynestrenol (LYN), and norethynodrel. Levonorgestrel (LNG) and norgestrel (NG) correspond to second generation progestagens and third generation progestagens are desogestrel (DSG) and its active metabolite etonogestrel, gestodene (GSD), and norgestimate (NGM) and

its active metabolite norgestromin (NGMN). Examples of other progestagens used in hormonal contraceptives are cyproterone acetate (CPA), chlormadinone acetate, nomegestrol, drospirenone (DRSP) and medroxyprogesterone acetate (MPA). These last progestagens are classified as pregnanes based on their structure. Estranes are comprised of the first generations progestagens, while the second and third generation progestagens belong to the gonanes.

Steroid hormones can be administered via different routes or applications, such as orally (pill), intrauterinely (intrauterine device (IUD)), transdermally (patch), subcutaneously (injectable or implant), or transvaginally (ring). The most commonly used route for combined formulations is orally and occasionally transdermally or transvaginally. Progestagen-only formulations are administered orally (mini pill) as well as subcutaneously or intrauterinely.

Combined hormonal contraceptives Several large studies[12–15] in the 1990s have confirmed the two-fold to four-fold increase in risk of venous thrombosis associated with oral contraceptive use, which was already shown in four studies[2–5] from the late 1960s. The risk of venous thrombosis is the highest in the first three months of combined oral contraceptive use, i.e., about twelve-fold increased compared with non-users[16–18]. With extended use the risk decreases to an approximately five-fold increased risk. Because the estrogen compound in combined oral contraceptives was thought to cause the increased risk of venous thrombosis, the dose of estrogen has been lowered from 150-100 µg after the introduction of the oral contraceptive to 50 µg in the 1960s to 30-35 µg and 20 µg in the 1970s[19,20]. The lower dose of ethinylestradiol in combined oral contraceptives was associated with a reduction in venous thrombosis risk[12,21–24]. The currently prescribed combined oral contraceptives containing 30 µg of ethinylestradiol are associated with a higher risk of venous thrombosis than contraceptives containing 20 µg[17,18].

Besides adjustments in the dose of ethinylestradiol, the progestagen compound was changed to reduce side effects of oral contraceptive use. After the introduction of the third generation progestagens as part of the combined oral contraceptives in the eighties, the risk of venous thrombosis among users of those compounds was investigated. It was shown that third generation oral contraceptive users have a higher risk of venous thrombosis compared with second generation users[8,16–18]. However, these results were disputed by reasoning that bias or confounding could explain the difference in risk between these progestagens. These issues were addressed in an opinion article[25] and a meta-analysis[16] in which it was shown that the presence of bias or confounding could not explain the observed results. Other progestagens have been developed after the introduction of the third generation progestagens, e.g., drospirenone (introduced in 2001) and dienogest (introduced in 1995). The use of drospirenone in a combined oral contraceptive has been shown to increase the risk of venous thrombosis[17,18] compared with non-use and compared with second generation contraceptive use[26,27]. No information concerning the risk of venous thrombosis is available for the contraceptive containing dienogest because this contraceptive is mainly prescribed in Germany[28].

To diminish the risk of venous thrombosis, it was attempted to prevent the first-pass effect of steroids. Because of oral intake of steroid hormones, the metabolism in the liver was thought to be important in the pathogenesis of venous thrombosis. After a drug is ingested, it is absorbed by the digestive system and enters the liver through the hepatic portal system[29]. The liver metabolizes many drugs before they enter the remaining circulation. The first-pass through the liver may greatly affect the bioavailability of an ingested drug[29], hence the name first-pass metabolism. This led to the hypothesis that transdermal and transvaginal administration of estrogens and progestagens may reduce venous thrombosis risk. The application of hormones via the skin or vagina bypass the first-pass effect in the liver be-

cause the hormones are first distributed to other organs and later, diluted, to the liver[30].

The information concerning the risk of venous thrombosis with vaginal ring or transdermal patch use is lacking due to their fairly recent introduction onto the market in 2001 and 2002, respectively and due to a limited number of users of these types of contraceptives. However, two case reports concerning mesenteric vein thrombosis[31] and cerebral venous sinus thrombosis[32] were reported in two vaginal ring users suggesting a potential association between vaginal ring use and thrombosis. Furthermore, a deep vein thrombosis was reported as a serious adverse event in vaginal ring users in each of two trials (of which one randomized controlled trial (RCT)) evaluating the efficacy and tolerability of the vaginal ring compared with a second generation combined oral contraceptive[33,34]. The FDA showed in a large cohort study that vaginal ring use increased the risk of venous thrombosis compared with combined oral contraceptive users[35]. This result was confirmed by another large cohort study conducted in Denmark[36].

The risk of venous thrombosis in transdermal patch users has been assessed in two observational studies using health insurance databases. Both studies were supported by the manufacturers. The first study reported no difference in venous thrombosis risk between transdermal patch users and users of a third generation combined oral contraceptive[37]. The second study, published one year later, reported an increased risk of venous thrombosis in patch users compared with third generation oral contraceptive users[38]. These results were confirmed in updated analyses of both studies[39,40]. After these results the US Food and Drug Administration (FDA) issued the following warning on 22 January 2008: "FDA believes that Ortho Evra is a safe and effective method of contraception when used according to the labeling, which recommends that women with concerns or risk factors for serious blood clots talk with their health care provider about using Ortho Evra versus other contraceptive option"[41]. In 2011,

the FDA performed their own study and showed that the use of the transdermal patch increased the risk of venous thrombosis compared with combined oral contraceptive users[35]. This result was confirmed in a large cohort study from Denmark[36].

In summary, the use of combined hormonal contraceptives is associated with an increased risk of venous thrombosis whether the hormones are administered in a pill, in a vaginal ring or in a transdermal patch.

Progestagen-only contraceptives Injectable progestagen-only contraceptives were developed in the late 1950s and early 1960s as a result of a growing understanding of steroid hormones and the research into oral contraceptives. In the late 1960s, the first oral progestagen-only contraceptive was developed when concerns were raised about the side-effects of combined oral contraceptives. Progestagen-only implants were developed in 1960s and 1970s and the hormone-releasing IUD was developed in the early 1970s.

Four case-control studies have assessed the risk of venous thrombosis associated with progestagen-only pills (POP) for contraceptive use: three studies[23,42,43] reported a potential increase in risk compared with either non-users or users of combined oral contraceptives containing levonorgestrel, whereas one study[44] reported a decrease in risk compared with non-users. The risk of venous thrombosis was not separately assessed per type of progestagen. A cohort study reported no increased risk of venous thrombosis in users of levonorgestrel or norethisterone or in users of desogestrel, although a relatively small number of women were using these contraceptives[17].

Information about the risk of venous thrombosis in implant users is lacking, probably due to the low number of women using this type of contraceptive. A large cohort study showed that the use of an implant was associated with an increased risk of venous thrombosis compared with non-use[36]. Three studies[42,45,46] have investigated the use of injectables containing MPA and all three

reported an increased risk of venous thrombosis relative to non-users. Regarding IUD use, two observational studies showed that the use of an IUD containing levonorgestrel was not associated with venous thrombosis[17,45].

In a nested case-control study, the risk of venous thrombosis was not increased in users of progestagen-only contraceptives (i.e., POP, injectable, and implant combined) compared with non-users[47]. Venous thrombosis risk per type of administration was not evaluated.

Currently, no definitive conclusion on the risk of venous thrombosis associated with progestagen-only contraceptives can be drawn due to variation in the progestagen used, the dose and mode of administration, and because a small number of women use progestagen-only contraceptives. However, there is an indication that users of IUD containing levonorgestrel or users of oral levonorgestrel have the same risk of venous thrombosis as non-users, whereas users of injectable methoxyprogesterone acetate have an increased risk.

Hormone replacement therapy

Hormone replacement therapy (HRT) is mainly administered to relief hot flushes caused by diminishing estrogen levels as a result of failing ovaries (e.g., premature ovarian failure or surgically caused menopause) or physiological menopause[48]. Estrogen-only HRT is prescribed to women without a uterus. In women with a uterus, the estrogen compound is combined with a progestagen as progestagen is needed to shed the developed endometrium (when progestagen is sequentially administered) or to prevent endometrial hyperplasia caused by estrogen (when progestagen is continuously administered). Estrogens or the combination of estrogen and progestagen are administered orally or transdermally via a patch. An estrogen implant can be used as well, although it is not commonly applied. Besides the relief of symptoms of menopause, HRT was thought to prevent osteoporosis

and cardiovascular diseases. It was hypothesized that the risk of osteoporosis and cardiovascular diseases were due to the drop in estrogen levels caused by menopause and that the risks could be prevented by replenishing estrogen with HRT.

Combined and estrogen-only administration In 1895, it was suggested that ovarian secretions could be used to treat ovarian failure and in 1896 the first therapeutic interventions were reported[49]. Over the course of the next thirty years, the ovarian hormones were identified, i.e., estrone, estriol, estradiol and progesterone. In the 1930s estrogen was used as a therapy in women with premature menopause (onset of menopause before the age of 40)[50]. Use of estrogen-only HRT became widespread in the 1960s and 1970s. However, in the 1970s it was shown that women with an intact uterus using estrogen-only therapy were at an increased risk for endometrial cancer[51]. Thereafter, combined HRT was given to women with a uterus and estrogen-only to women without a uterus[52]. Since then many observational studies were conducted to establish whether HRT was protective against cardiovascular diseases. A meta-analysis of observational studies published in 1991 showed that the use of HRT was protective against cardiovascular diseases[53]. However, the hormones used and dose and the mode of administration was different across the studies making a comparison across studies difficult. Furthermore, women taking HRT may be different from women not taking HRT which can influence the results obtained in observational studies. These issues are reduced in trials where women are randomized to receive HRT or a placebo.

The HERS study[54] (Heart and Estrogen/progestin Replacement Study) was one of the first trials showing that HRT use did not reduce the rate of secondary coronary heart disease. In this RCT, women with established coronary disease were randomized to receive either combined HRT or a placebo. In a Cochrane review from 2005[55], the prevention of cardiovascular disease in postmenopausal women using HRT was assessed. A total of ten

RCTs were included of which two trials included healthy women and eight trials included women with heart disease. No protective effect of HRT was seen for any of the arterial outcomes assessed. One of the secondary outcomes evaluated was venous thrombosis. Combined oral HRT (i.e., conjugated equine estrogens (CEE) with MPA) was associated with a twofold increased risk of venous thrombosis. One trial used 17β-estradiol with norethisterone and also found an increase in risk[56]. Estrogen-only oral HRT (i.e., 17β-estradiol) was not associated with venous thrombosis; however, only two RCTs (i.e., SPRIT 2002[57] and WEST[58] trial) contained data on estrogen-only HRT. The Women's Health Initiative (WHI) group conducted the WHI Conjugated Equine Estrogen (CEE) trial published in 2006[59,60] (not included in the aforementioned Cochrane review) and evaluated the use of estrogen-only oral HRT on major disease incidence rates. The results from this WHI CEE trial[60] were that the use of estrogen-only oral HRT increased the risk of venous thrombosis although less pronounced than with the use of combined oral HRT (evaluated in the WHI E+P trial[61]). The results from the WHI CEE trial are in contrast to the results from the SPRIT 2002 and WEST trials which can be explained by the small number of events in the last two trials (a total of 9 and 7 events, respectively) compared with 179 events in the WHI CEE trial. Furthermore, both the SPRIT 2002 and WEST trial used 17β-estradiol as estrogen and included women with a first myocardial infarction (SPRIT 2002 trial) or women with an ischemic stroke or transient ischemic attack (WEST trial), whereas the WHI CEE trial used CEE as estrogen and included healthy women without a uterus. Besides data from RCTs, observational studies have also shown that HRT use was associated with venous thrombosis. A matched case-control study[62] showed that all types of HRT were associated with a 3.5-fold increased risk for venous thrombosis compared with non-users. A nested case-control study[63] confirmed these results showing that HRT use increased the risk of venous thrombosis 2.1-fold compared with non-users. Data col-

lected from observational studies and RCTs regarding HRT use and the risk of venous thrombosis were summarized in a meta-analysis. Both data from observational studies and RCTs showed a twofold increased risk for venous thrombosis when using oral HRT[64].

In addition to 17β-estradiol and CEE, esterified estrogens can also be used in HRT. Like CEE, esterified estrogens are a combination of naturally occurring estrogens and their conjugates, but in different relative amounts. In a case-control study[65], the risk of venous thrombosis with the use of esterified estrogens and CEE with or without MPA in HRT has been evaluated. Compared with non-users, the use of esterified estrogens with or without MPA did not increase the risk of venous thrombosis. Among hormone users, the use of CEE with or without MPA increased the risk of venous thrombosis in comparison to users of esterified estrogen without MPA.

No RCTs were conducted with HRT administered through a patch. In total four studies, i.e., three case-control studies including the ESTHER study[62,63,66,67] and one cohort study (the E3N cohort study)[68] evaluating the risk of venous thrombosis with HRT use provided data on the thrombotic risk of transdermal administered steroid hormones. Two case-control studies[62,63] reported that after adjustment for multiple risk factors the risk of venous thrombosis was increased with the use of transdermally administered HRT. However, no information was provided on the type of estrogen and whether in addition to estrogen, also a progestagen was supplied. The ESTHER study and the E3N cohort study, both conducted by the same research group, reported no increased risk for venous thrombosis in transdermal HRT users. In these studies, mostly 17β-estradiol was used. It is unclear whether this was estrogen only or combined with a progestagen. However, in an earlier publication of the ESTHER study[67] the addition of a progestagen to transdermal 17β-estradiol did not influence the risk of venous thrombosis.

In summary, the use of combined oral HRT is associated with

an increased risk of venous thrombosis. For estrogen-only oral HRT there is a strong indication that this is associated with an increased thrombotic risk as well. With regard to transdermal administered HRT, no firm conclusions can be drawn because only a small number of women used this type of administration.

Other sex hormone applications

Growth inhibition Tall stature is defined as the height of an individual two standard deviations above the corresponding mean height for a given age, sex and population group [69]. The strongest increase in height occurs when estradiol levels are low, although a direct relationship between estradiol and growth hormone levels is not yet established [69]. Sex steroids, estrogens for females and androgens for males, are used to limit the expected height in tall children. High doses of gonadal steroids, especially estrogens, accelerate bone maturation. In tall girls, 100-200 µg of ethinylestradiol is continuously orally administered and in the last 7-10 days of each month together with a progestagen to shed the developed endometrium. Since higher doses of the same hormones as in combined hormonal contraceptives are used, the question is whether the treatment for tall girls is also associated with an increase in risk of venous thrombosis.

Venous thrombosis during hormone treatment for tall stature has been reported only sporadically [70,71], and all such cases have occurred in a clinical situation involving an elevated risk for venous thrombosis, e.g., immobilisation or surgery. Venous thrombosis is much less common in children (i.e. 1 in 100,000 person per year) than in adults (i.e. 1 in 1,000 persons per year), and any venous thrombosis event in this young age group is usually due to a combination of multiple inherited and acquired risk factors [72].

Sex change Transsexuals denote individuals who desire to live permanently as a member of the opposite sex and who want to

undergo sex reassignment. To this end, transsexuals receive hormone therapy for life. Female-to-male transsexuals receive androgens to induce male body features, whereas male-to-female transsexuals receive either gonadotrophin-releasing hormone (GnRH) agonists or progestational compounds (e.g., CPA) to suppress the original male sex characteristics and subsequently ethinylestradiol (100 µg per day) to induce female body features. The dose of progestagen is about fifty-fold higher and the estrogen dose is about three times higher than in combined hormonal contraceptives. One of the adverse events of this hormone therapy is venous thrombosis. The occurrence of venous thrombosis in male-to-female transsexuals receiving oral progestagen and estrogen was 45-fold increased compared to the general population[73]. Because of the high incidence of venous thrombosis during this study, the administration route was changed from oral to transdermal in male-to-female transsexuals over the age of 40. The corresponding occurrence of venous thrombosis was still twenty-fold increased compared with the general population[74]. Of the 36 unprovoked cases, 21 transsexuals experienced a venous thrombosis in their first year of hormone therapy resembling the same risk pattern as in combined oral contraceptive users.

In conclusion, the use of orally administered hormones to induce female body characteristics in male-to-female transsexuals is associated with an increased risk of venous thrombosis.

Towards a mechanism of combined oral contraceptive induced venous thrombosis

Hypothesis

After reviewing the literature regarding steroid hormone use and venous thrombosis, we summarize several associations. The use of combined oral contraceptives is associated with an increased

risk of venous thrombosis. Compared with combined oral contraceptives containing a second generation progestagen (i.e., levonorgestrel), third generation contraceptives and contraceptives containing other non-classified progestagens induce a higher risk of venous thrombosis. Furthermore, the dose of ethinylestradiol in combined oral contraceptives is positively and monotonously gradedly associated with the risk of venous thrombosis. Regarding contraceptives containing solely progestagen, only associations concerning progestagens levonorgestrel and MPA can be summarized. Levonorgestrel administered orally or intrauterinely without ethinylestradiol is not associated with venous thrombosis, whereas the progestagen MPA administered via injection increases the risk. Finally, the use of combined and estrogen-only HRT administered orally increases the risk of venous thrombosis as well. In general, the use of orally administered synthetic sex steroid hormones (combined therapy either for contraceptive use or for HRT) is associated with an increased risk of venous thrombosis (Table 1.1).

Regarding the association between oral contraceptives and venous thrombosis, the progestagen levonorgestrel seems to have a unique role. Combined with ethinylestradiol, levonorgestrel users are at an increased risk of venous thrombosis, but not as much as users of a third generation progestagen or other non-classified progestagens, whereas the sole use of levonorgestrel (i.e., IUD or oral) does not increase the risk of venous thrombosis. Furthermore, levels of ethinylestradiol over 24 hours are lower in levonorgestrel users than in desogestrel users while receiving the same dose of ethinylestradiol (30 µg) [75]. Overall, levonorgestrel appears to be able to modify the effect caused by ethinylestradiol, whereas third generation progestagens seem to lack this ability.

Based on the literature it is difficult to determine whether estrogen, progestagen or combination of both is pivotal in the pathogenesis of venous thrombosis. However, based on the association between ethinylestradiol dose in combined oral contraceptives and venous thrombosis, it is likely that ethinylestradiol

Table 1.1: The risk of venous thrombosis for different applications of sex steroid hormones in users versus non-users

Application **Progestagen**	**Oral**	**Patch 3rd**	**Ring 3rd**	**Injectable MPA**	**IUD LNG**	**Implant 3rd**
Contraceptive use						
EE + P	+	+	?	NA	NA	NA
P	-/+*	NA	NA	+	-/+	?
HRT use						
E + P	+	?	NA	NA	NA	?
E	+	?	NA	NA	NA	?
Growth restriction						
EE + P	?	NA	NA	NA	NA	NA
Sex change (♂ → ♀)						
EE + P	+	?	NA	NA	NA	NA

EE, ethinylestradiol; E, estrogen; P, progestagen; NA, not applicable; +, increased risk of venous thrombosis; -/+ no association with venous thrombosis; -, decreased risk of venous thrombosis; ?, no data available

* No increased risk in levonorgestrel users compared with non-users

plays a role in the pathogenesis of venous thrombosis. The question is how ethinylestradiol leads to an increased risk of venous thrombosis. Because ethinylestradiol is a synthetic hormone and can be orally administered, the first-pass metabolism in the liver may play an important role. The first-pass metabolism in the liver is known to influence the bioavailability of many synthetic drugs. Several coagulation factors are produced in the liver making it likely that ethinylestradiol can influence the production of coagulation factors. However, the use of a transdermal patch or a vaginal ring that bypass this first-pass metabolism, is also associated with an increased risk of venous thrombosis suggesting that the first-pass metabolism of ethinylestradiol may not be the sole player in the pathogenesis of venous thrombosis. A study

reported that the area under the curve (AUC) of ethinylestradiol levels over 24 hours in vaginal ring and transdermal patch users are larger than with oral administration [76]. Therefore, total levels of ethinylestradiol may play a role as well.

In the Netherlands, the combined oral contraceptive is the most popular birth control method making the risk of venous thrombosis a realistic concern. Currently, it is still unclear how combined oral contraceptives, in particular ethinylestradiol, can cause venous thrombosis. The focus of this thesis is on the role of ethinylestradiol in the pathogenesis of venous thrombosis in premenopausal women.

Effects on coagulation and markers of coagulation or venous thrombosis

In 2005, the European Medicines Agency (EMA) provided an updated guideline on clinical investigation of newly developed steroid contraceptives in women to establish the contraceptive efficacy and to describe the risks and adverse events of the new contraceptive [77]. Regarding the safety of the contraceptive concerning venous thrombosis, the EMA suggested measuring the following haemostatic variables: prothrombin fragment 1+2 levels, D-dimer levels, factor VII, factor VIII, factor II, antithrombin, protein S and protein C. Besides these individual coagulation factors, measuring activated protein C (APC) resistance (ETP-based, APTT-based) was recommended by the EMA as well. APC resistance is the relative inability of protein C to cleave activated factor V or activated factor VIII leading to a prothrombotic state. Both APC resistance assays use different triggers, measure different endpoints and are influenced by different determinants; therefore, both assays provide insights into different mechanisms of APC resistance [78]. Further, EMA recommended to measure sex hormone binding globulin (SHBG) levels as an indicator of the hormonal activity of the contraceptives. These

individual coagulation factors, APC resistance and SHBG levels suggested by the EMA will be evaluated regarding the use of ethinylestradiol in contraception, restriction of tall stature and sex change. HRT use will not be assessed because ethinylestradiol is not used in HRT.

Individual coagulation factors The use of combined hormonal contraceptives influenced many coagulation factors (Table 1.2). Use of combined oral contraceptives increased factors involved in coagulation and fibrinolysis as well as some factors in anticoagulation, whereas other anticoagulation factors were decreased[79–81]. In third generation combined oral contraceptive users, the increase was more pronounced than in second generation users[79–81]. For instance, the increase in factor VII and the decrease in protein S concentrations were more pronounced in third generation oral contraceptive users.

The use of a vaginal ring containing a third generation progestagen showed the same effects on coagulation factors as combined oral contraceptives with third generation progestagens[82,83]. Compared with second generation combined oral contraceptive users, vaginal ring users showed a more pronounced increase in factor VII and a more pronounced decrease in functional protein S levels[83]. However, another study[82] reported that functional protein S levels were not influenced by the vaginal ring, although in this study women were able to choose their contraceptive, i.e., either a vaginal ring or a second generation combined oral contraceptive. As a consequence, the patient characteristics can influence these results because they may be different between users of a vaginal ring or a combined oral contraceptive.

The following coagulation factors were measured in transdermal patch (containing a third generation progestagen) users[84]: prothrombin fragment 1+2, antithrombin, and protein S concentrations. The effect on these coagulation factors were the same as in third generation combined oral contraceptive users with a more pronounced decrease in protein S concentrations. However,

Table 1.2: Effect on markers of venous thrombosis in sex steroid hormones versus non-users

Application **Progestagen**	**Oral**	**Patch** **3rd**	**Ring** **3rd**
Separate factors			
Coagulation			
F1+2	+	+	+
Factor II	+	?	?
Factor VII	+	?	+
Factor VIII	+	?	+
Anticoagulation			
Antithrombin	-	-	-/+
Protein C	+	?	+
Protein S	-	-	-/+
Fibrinolysis			
D-dimer	+	+	+
APC (ETP-based)			
APC resistance	+	+	+
Hormonal activity			
SHBG	+	+	+

APC, activated protein C; SHBG, sex hormone binding globulin; +, increased levels/activity; -/+ no change in levels/activity; -, decreased levels/activity; ?, no data available

no other studies evaluated the effect of transdermal patch use on coagulation factors.

Treatment for growth inhibition in tall girls (i.e., ethinylestradiol with a progestagen added in the last days of the cycle) lowered protein S concentrations[85] and functional antithrombin levels[85–87], and increased functional protein C levels[85] and prothrombin 1+2 concentrations[85]. Another study[88] showed that

factor II concentrations were increased in tall girls, whereas no effect was observed for factor VII, factor VIII and antithrombin concentrations. However, in this study only eight girls were included and compared to controls while the other studies compared the effect before treatment with the effect during treatment in a larger number of girls. Overall, treatment for growth inhibition had the same effects on coagulation factors as the use of combined oral contraceptives. Although the magnitude of the effects was difficult to assess because no comparison was made with a combined oral contraceptive.

Only functional protein C and protein S levels and factor II concentrations were measured in male-to-female transsexuals receiving ethinylestradiol and CPA. Male-to-female transsexuals had lower protein S concentrations and slightly higher functional levels of protein C than their baseline measurements[89]. No association between changes in coagulation and changes in hormone levels of 17β-estradiol, testosterone, LH and FSH was observed suggesting that the effect on coagulation is a sole effect of ethinylestradiol. No other studies evaluated the effect of this hormone therapy or any other coagulation factors.

The effects on coagulation factors in combined oral contraceptive users have been extensively researched showing that the use influenced levels of many coagulation factors. Few studies researched the effects of hormone use for contraception administered via a ring or patch, restriction of tall stature or sex change on coagulation factors, but from the studies that were conducted, it is likely that these applications can influence levels of coagulation factors.

Activated protein C (APC) resistance As mentioned before, activated protein C resistance can be measured in two ways; APTT-based or ETP-based. When the two methods were compared with regard to the effect of combined oral contraceptive use, the effect on the ETP-based APC resistance was more pronounced than on the APTT-based test[90]. Furthermore, third generation

combined oral contraceptives users showed a more pronounced increase in APC resistance (ETP-based) than second generation users providing a measure for the difference in venous thrombosis risk between these generations. For these reasons the ETP-based APC resistance test will only be taken into account.

The APC resistance predicts venous thrombosis risk in men and women, as well as in oral contraceptive users and non-users [91]. Several other studies confirmed that APC resistance was increased in combined oral contraceptive users [81,83,84] and that the effect was more pronounced in users of a third generation progestagen than with a second generation progestagen [81]. Both in users of a vaginal ring and a transdermal patch, the APC resistance was increased as well [83,84]. Compared with second generation combined oral contraceptive users, the increase in APC resistance was more pronounced in vaginal ring users [83]. The APC resistance increased appreciably with the use of a patch compared with a third generation combined oral contraceptive [84]. No data was available on girls receiving sex hormones for tall stature and the effect on APC resistance. In the male-to-female transsexuals receiving ethinylestradiol with CPA, the APC resistance was increased compared to baseline [89]. Overall, the use of ethinylestradiol with a progestagen led to the activation of coagulation in women using contraceptives administered via a pill, a vaginal ring or a transdermal patch, or in male-to-female transsexuals.

Sex hormone binding globulin (SHBG) Sex hormone binding globulin (SHBG) is a plasma glycoprotein, primarily produced in hepatocytes, that binds androgens and thereby regulates their bioavailability. SHBG levels vary due to multiple regulating factors such as age, body weight, sex steroids, or insulin. Estrogens like ethinylestradiol are able to increase the production of SHBG, whereas progestagens induce a decrease of SHBG levels depending on the type and dose [92,93]. Therefore, the effect of combined oral contraceptives on SHBG levels can be seen as the sum of

the stimulating effect of ethinylestradiol and the inhibiting effect of the progestagen resulting in the total estrogenicity of a contraceptive[93]. This estrogenicity of a contraceptive can influence the venous thrombosis risk[93]. A positive correlation was observed between SHBG levels and APC resistance supporting the hypothesis that SHBG levels can be seen as a marker for venous thrombosis[93,94]. However, SHBG levels have not yet been researched in association with venous thrombosis risk.

In general, combined oral contraceptive use was associated with an increase in SHBG levels and this increase was more pronounced in third generation progestagen users than in second generation users[95,96]. The use of a vaginal ring[83] or a transdermal patch[84] was also associated with an increase in SHBG levels. Compared with a second generation combined oral contraceptive, the SHBG levels were higher in both vaginal ring and transdermal patch users[97]. The SHBG increase was more pronounced in transdermal patch users compared to vaginal ring users[97]. No information was available on SHBG levels and hormone use for tall stature or sex change.

Data presented here confirmed that combined oral contraceptive users, vaginal ring users and transdermal patch users have an increased risk of venous thrombosis.

Experimental data on ethinylestradiol

Several epidemiological studies established that the use of combined oral contraceptives increased the risk of venous thrombosis. The use of contraceptives influenced the levels of coagulation factors and increased APC resistance and SHBG levels. Data from experimental research may provide further evidence on the mechanism how ethinylestradiol can influence the risk of venous thrombosis.

Ethinylestradiol on a cellular level Ethinylestradiol is able to bind to the estrogen receptor. This complex of ethinylestradiol

and estrogen receptor was able to function as a transcription factor, to enter the nucleus and to bind to estrogen response elements (ERE) in the DNA[98]. This resulted in transcriptional activation of nearby genes. Many coagulation factors genes contain a ERE suggesting that ethinylestradiol is able to influence coagulation factors levels directly.

17β-Estradiol and ethinylestradiol were compared with regard to their ability to translocate the estrogen receptor to the nucleus of hepatocytes[99]. 100-fold higher concentrations of 17β-estradiol was needed to lead to the same promotion of translocation as ethinylestradiol in parenchyma cells in the liver of rats. Furthermore, they also compared the metabolic pathways and found that 17β-estradiol was much more metabolized than ethinylestradiol. In the metabolism of 17β-estradiol, one major route[100] is the oxidation at the C-17 position which is blocked by the ethinyl group in ethinylestradiol at the same C-17 position (Figure 1.1). This potentially explains why 17β-estradiol was more metabolized than ethinylestradiol.

First-pass metabolism of ethinylestradiol An overview of the first-pass metabolism of ethinylestradiol is given in figure 1.2.

One of the enzymes involved in the ethinylestradiol first-pass metabolism in the liver is cytochrome p-450 3A4 (CYP3A4) which hydroxylates ethinylestradiol to hydroxy-ethinylestradiol. Several studies looked at the effect of ethinylestradiol and different progestagens on the content of cytochrome P-450 enzymes in the liver and the inhibition of CYP3A4[101–103]. Both ethinylestradiol and gestodene (third generation progestagen) were able to reduce the total content of this enzyme family in the liver with at least 30% and to inhibit the enzyme CYP3A4. However, a higher dose of ethinylestradiol and gestodene was used in this study than the dose used in combined oral contraceptives and the combination of ethinylestradiol and gestodene was not evaluated so the question remains whether the currently used dose of ethinylestradiol in combined oral contraceptives and the

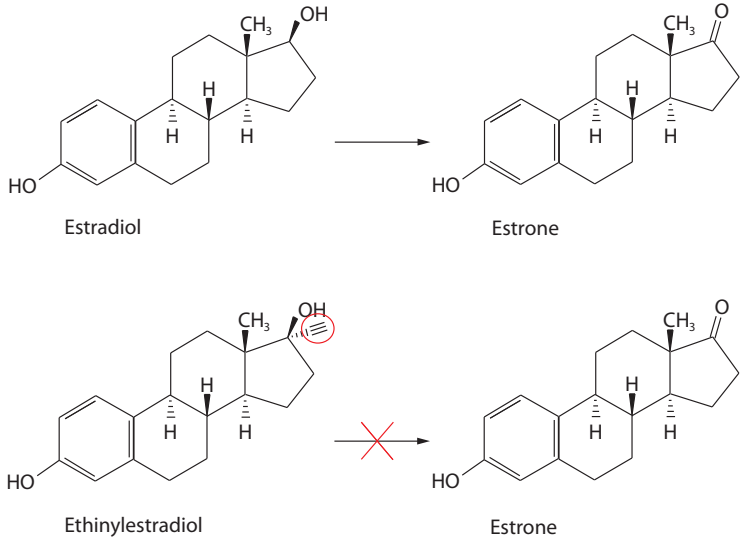

Figure 1.1: The oxidation at C-17 of estradiol to estrone is shown in the top part. This oxidation is blocked by the ethinyl-group in ethinylestradiol (indicated by circle) depicted in the bottom part

combination with gestodene is also able to inhibit the enzyme CYP3A4 in vivo.

Taken together the data from experimental research provides further insights into how ethinylestradiol can influence the venous thrombosis risk.

Research questions

Biochemical aspects

Ethinylestradiol levels vary according to the day in the cycle and the time since last oral contraceptive pill[104]. For an accurate

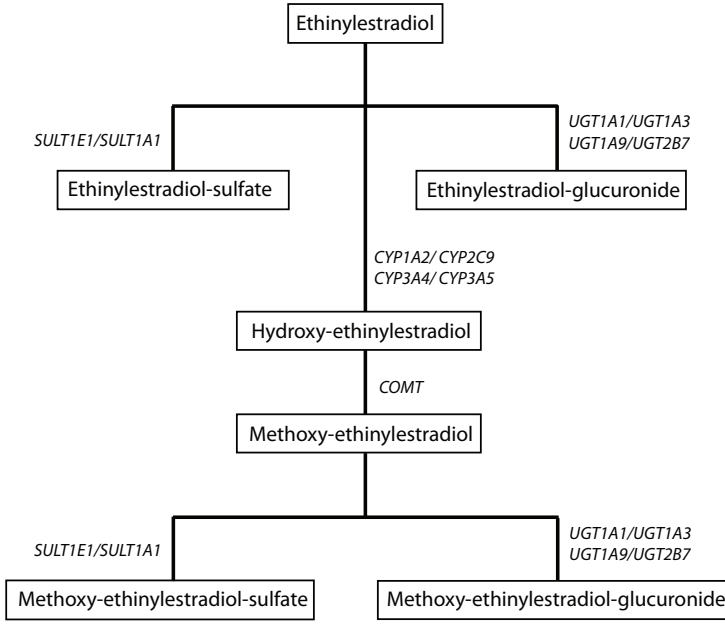

Figure 1.2: The first-pass metabolism of ethinylestradiol in the liver. Genes involved in this metabolism are depicted in italics

measurement, blood has to be drawn at the same moment for every woman included in a study. However, in current case-control studies of venous thrombosis, blood is taken at random in the menstrual cycle and after the thrombotic event when many women have stopped using combined oral contraceptives. In most research settings regarding venous thrombosis ethinylestradiol levels are not commonly measured. No study has assessed the association between the levels of ethinylestradiol and venous thrombosis risk.

Because SHBG levels are seen as a marker for the estrogenicity of combined oral contraceptives and venous thrombosis risk

and are not affected by daily fluctuations, SHBG levels will be measured instead of ethinylestradiol levels. To determine whether SHBG levels are a risk factor for venous thrombosis, the association between increased SHBG levels and the risk of venous thrombosis in women not using hormonal contraceptives will be discussed in **chapter 2**. The association between the dose of ethinylestradiol in combined oral contraceptives and SHBG levels will be discussed in **chapter 3**.

First-pass metabolism of ethinylestradiol To determine whether genetic variation in the first-pass metabolism of ethinylestradiol can at least in part explain the risk of venous thrombosis in oral contraceptive users, common genetic variation in enzymes in this metabolism will be investigated. Many genes and their enzymes are involved in the first-pass metabolism of ethinylestradiol (Figure 1.2). Genes are selected based on their ability to convert ethinylestradiol and on their expression in the liver. Genetic variation in the selected genes will be assessed through haplotypes. A haplotype is a combination of alleles on a chromosome that is not affected by recombination and consequently transmitted together. Because of this linkage, without actual measurement, a known single nucleotide polymorphism (SNP) can provide information about neighbouring SNPs.

Conjugation and hydroxylation are the first two steps in the first-pass metabolism of ethinylestradiol. Sulfonation and glucuronidation are both conjugation steps leading to inactive and water-soluble ethinylestradiol that can easily be excreted through the urine or bile. The genes *SULT1A1* and *SULT1E1* code for sulfotransferases that are involved in sulfonation[105–110] of ethinylestradiol and the genes *UGT1A1*, *UGT1A3*, *UGT1A9* and *UGT2B7* code for UDP-glucuronosyltransferases involved in the glucuronidation[110–113]. Hydroxylation and subsequent methylation, of the hydroxyl group, lead to hydroxy-ethinylestradiol and methoxy-ethinylestradiol, respectively. To inactivate these hormones, the aforementioned conjugation steps are repeated.

The genes *CYP1A2*, *CYP2C9*, *CYP3A4* and *CYP3A5* code for enzymes involved in the hydroxylation step[102,110,114,115] and the *COMT* gene codes for catechol O-methyltransferase involved in the methylation step[116]. Genetic variation in these genes, their effect on SHBG levels and the association with venous thrombosis will be discussed in **chapter 4**.

Clinical aspects

Current guidelines advice women to refrain from using combined hormonal contraceptives after a venous thrombosis. Progestagen-only contraceptives can be used. Adherence to these guidelines and potential explanations will be assessed in **chapter 5**.

Combined oral contraceptive use increases the risk of a first venous thrombosis, whether contraceptive use is also associated with a second event is unclear. To date, one study assessed the risk of a recurrence and hormonal contraceptive use[117]. A total of 14 recurrences were observed among premenopausal women exposed to hormonal risk factors (oral contraceptive use or pregnancy) of which 11 occurred in women using hormonal contraceptives. The incidence of a recurrence was 4.3 times higher in hormonal contraceptive users than in non-users (incidence rate ratio (IRR) 4.3, 95%CI: 1.7 to 11.1). We will analyse the incidence rate of recurrent venous thrombosis in premenopausal women and the effect of hormonal contraceptives used at the first or second event in **chapter 6**. The effect of oral and non-oral preparations on recurrent venous thrombosis will be discussed as well.

Although it has been shown that there is a difference in the risk of a first venous thrombosis per generation of progestagens, no clear overview of the associations between different combined oral contraceptives and the risk of venous thrombosis exists. We set out to provide an overview of the risk of a first venous thrombosis per combined oral contraceptive preparation. A network meta-analysis will be performed because combined oral

contraceptives are mostly compared to non-use or to a combined oral contraceptive containing levonorgestrel with 30 µg ethinylestradiol resulting in gaps in direct evidence. The results of the network meta-analysis will be discussed in **chapter 7**.

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